Well Pollutants Identified With A New Mass Spectrometric Technique

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1. ABSTRACT

The incidence of cancer observed in children living near a contaminated site in New Jersey was higher than normal. Trace amounts of several isomeric compounds were detected by GC/MS in a concentrated extract of water taken from a well that serviced 50,000 people. Mass Peak Profiling from Selected Ion Recording Data (MPPSIRD) provided the sensitivity and scan speed necessary to acquire mass peak profiles at mass resolutions of 10,000 to 20,000 for the molecular ion (M++) and 10 fragment ions as capillary GC peaks eluted. Exact mass differences between the molecular and fragment ions corresponded to unique combinations of atoms for the neutral losses observed in low resolution mass spectra. Identification of the neutral losses simplified mass spectral interpretation by limiting the number of possible structures for the fragment ions. After inspecting library mass spectra for smaller molecules, isomeric structures were hypothesized with cyano and alkylcyano groups attached to tetralin. A literature search found that six of these isomers were consistent with an industrial polymer synthesis performed by the party that generated the waste. The proposed structures were confirmed by mass spectra and GC retention times for three of the isomers in a standard provided from the polymerization process now used.

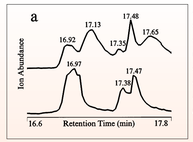
2. INTRODUCTION

The Reich Farm Superfund Site near Tom's River, NJ, is a relatively flat, sandy area of approximately three acres, surrounded by commercial establishments and woodland. In 1971, an independent waste hauler leased the site and dumped drums containing solvents, still bottoms, and residues from organic chemical manufacturing. More than 100,000 people residing within three miles of the site are served by nearby wells.

The NJ Department of Health and Senior Services reported that the incidence of cancer among children in the area was greater than expected. The NJ Department of Environmental Protection found traces of several isomeric compounds in a well that supplied 50,000 people. Neither they nor other laboratories were able to determine the elemental composition of the isomers using conventional mass spectrometric techniques. A concentrated extract was sent to the U.S. EPA's Environmental Sciences Division (ESD) for analysis. FTIR and NMR studies were precluded by the low level of the analytes in the extract.

Industrial chemical syntheses create a myriad of waste compounds. Most of these compounds are not found in mass spectral libraries. When analyzing chemicals dumped at a Superfund site located within 100 miles of numerous industries that generate waste, one does not know *a priori* which elements are contained in the compounds. For industrially generated organic compounds, C, H, O, N, P, S, Si, F, Cl, and Br should all be considered.

Shown in Figure 1a is an ion chromatogram for m/z 129, the base peak in the mass spectra. The mass spectrum for one isomer is shown in Figure 1b. No library matches were found with the Wiley library (262,000 entries) or the NIST library (62,235 entries).



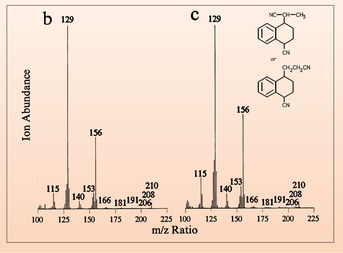


Figure 1. (a) m/z 129 ion chromatogram for well water extract (top) and standard (bottom); (b) mass spectrum of 4th isomer in the extract; and (c) mass spectrum of isomer in the standard with the same retention time.

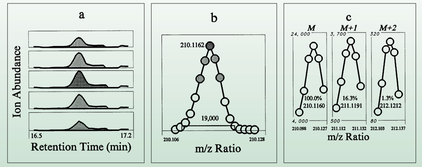
Fig. 1

3. Mass Peak Profiling from Selected Ion Recording Data (MPPSIRD)

MPPSIRD was developed by the ESD to determine the elemental compositions of environmental contaminants, a crucial step in their identification [1]. In Figure 2a are several ion chromatograms for m/z ratios that were monitored across a mass peak profile. The areas of the chromatographic peaks were among those plotted to provide the mass peak profile in Figure 2b. The exact mass was calculated as the weighted average of several points near the top of the profile. In Figure 2c are shown partial profiles plotted in the same way. Partial profiles were monitored by a single SIR descriptor to provide exact masses and abundances relative to the $M^{+\bullet}$ partial profile for the $(M+1)^{+\bullet}$ and $(M+2)^{+\bullet}$ partial profiles. SIR provided more than 100 times greater sensitivity than full scanning and 6 times faster cycle times at 20,000 resolution using a VG70-250SE double focusing mass spectrometer. Consequently, MPPSIRD provided exact masses and relative abundances of ions from major or trace components that reside in the mass spectrometer for short times. Data are routinely acquired at 20,000 resolution using capillary GC.

Fig. 2

Figure 2. Ion chromatograms (a) for m/z ratios corresponding to the 5 shaded points in (b), a full mass peak profile. In (c) 3 partial profiles are shown.



4. Determining the Correct Composition Using a Profile Generation Model (PGM)

A PGM calculated the possible elemental compositions that contained C, H, O, N, P, S, Si, and F atoms for 210.1160 Da ± 2.5 ppm, the exact mass of the presumed molecular ion, determined at 20,000 resolution. Cl and Br were not considered, because the isotopic abundance patterns associated with these elements were not observed in the mass spectra. The PGM calculated the exact masses and relative abundances of $(M+1)^{+\bullet}$ and $(M+2)^{+\bullet}$ partial profiles for each composition and applied error limit criteria to the experimental data to reject all but the correct one [2]. Finally, the PGM printed a table similar to Table 1 listing the 5 possible compositions, the mass defects, and relative abundances with an "X" beside each criterion that was failed. The trace levels of the analytes necessitated use of less than 20,000 resolution to study the $(M+1)^{+\bullet}$ and $(M+2)^{+\bullet}$ profiles.

Table 1. Elemental compositions and quantities used to select the correct one

m/z 210.1160 ± 2.5 ppm

Resolution:			20,000	10,000	5,000	10,000		5,000
#	RDB	Composition	M	M+1	M+2	%M+1 (Range)		%M+2 (Range)
1	1.0	$C_5H_{16}N_6PF$	.1158	.1177	.1190	7.2 (6.2- 8.4)	X	0.3 (0.2- 0.3) X
2	0.0	$C_6H_{20}N_4P_2$	.1163	.1187	.1214	8.3 (7.4- 9.3)	X	0.3 (0.2- 0.3) X
3	5.5	$C_9H_{13}N_5F$	.1155	.1181	.1204	11.3 (10.0-12.8)	X	0.6 (0.5- 0.7) X
4	4.5	$C_{10}H_{17}N_3P$	.1160	.1190	.1217	12.0 (10.8-13.4)	X	0.7 (0.6- 0.8) X
5	9.0	$C_{14}H_{14}N_2$	.1157	.1189	.1219	16.2 (14.7-17.7)		1.3 (1.1- 1.4)
Experimental:			.1160	.1187	.1208	16.3		1.3

5. Rejecting Functional Groups Based on the Elemental Composition

The nominal mass of the molecular ion indicates whether it contains an even or odd number of N atoms, but provides no other information about the functional groups in a molecule. The elemental composition of the m/z 210 ion, $C_{14}H_{14}N_2^{+\bullet}$, determined from high mass resolution data excluded alcohols, aldehydes, ketones, ethers, esters, carboxylic acids, peroxides, nitro-, nitroso-, or other oxygen, phosphorous, sulfur, halogen, or metal containing compounds. When the formula, $C_{14}H_{14}N_2$ was searched rather than the molecular weight of 210 Da, the number of mass spectra selected was reduced from 1285 to 33 for the Wiley library and from 342 to 13 for the NIST library. Although the mass spectra were not in the library, these reductions illustrate that knowledge of the elemental composition greatly reduced the number of possible compounds.

Table 2. Exact Masses for 10 Fragment Ions from the Isomers

Experimental Composition	Experimental Mass	Theoretical Mass	Error (mmu)	C, H & N Combinations with Same Nominal Mass		Errors (mmu)	
$C_{14}H_{12}N_2$	208.1001	208.1000	+0.1				
$C_{14}H_{10}N_2$	206.0846	206.0844	+0.2				
$C_{13}H_7N_2$	191.0607	191.0609	-0.2	$C_{14}H_9N$		-12.8	
$C_{13}H_{11}N$	181.0887	181.0891	-0.4	$C_{12}H_9N_2$	$C_{14}H_{13}$	+12.5	-12.6
$C_{12}H_8N$	166.0661	166.0657	+0.4	$C_{11}H_6N_2$	$C_{13}H_{10}$	+13.0	-12.2
$C_{11}H_{10}N$	156.0816	156.0813	+0.3	$C_{10}H_8N_2$	$C_{12}H_{12}$	+12.9	-12.3
$C_{11}H_7N$	153.0578	153.0578	0.0	$C_{10}H_5N_2$	$C_{12}H_9$	+12.5	-12.6
$C_{10}H_6N$	140.0501	140.0500	+0.1	$C_9H_4N_2$	$C_{11}H_8$	+12.7	-12.5
$C_{10}H_9$	129.0707	129.0704	+0.3	$C_8H_5N_2$	C_9H_7N	+25.4	+12.9
C_9H_7	115.0548	115.0548	0.0	$C_7H_3N_2$	C_8H_5N	+25.2	+12.6

Table 3. Exact Masses of Neutral Losses Determined from Mass Differences Between the Molecular and Fragment Ions

Experimental Atoms	Experimental Mass	Theoretical Mass	Error (mmu)	C, H & N Combinations with Same Nominal Mass		Errors (mmu)	
H_2	2.0159	2.0157	+0.2				
H_4	4.0314	4.0313	+0.1				
CH_7	19.0553	19.0548	+0.5		NH_5		+13.1
CH_3N	29.0273	29.0265	+0.8	C_2H_5	N_2H	-11.8	+13.3
C_2H_6N	44.0499	44.0500	-0.1	C_3H_8	CH_4N_2	-12.7	+12.5
C_3H_4N	54.0344	54.0344	0.0	C_4H_6	$C_2H_2N_2$	-12.6	+12.6
C_3H_7N	57.0582	57.0578	+0.4	C_4H_9	$C_2H_5N_2$	-12.2	+12.9
C_4H_8N	70.0659	70.0657	+0.2	C_5H_{10}	$C_3H_6N_2$	-12.4	+12.8
$C_4H_5N_2$	81.0453	81.0453	0.0	C_6H_9	C_5H_7N	-25.1	-12.5
$C_5H_7N_2$	95.0612	95.0609	+0.3	C_7H_{11}	C_6H_9N	-24.9	-12.3

Fig. 3

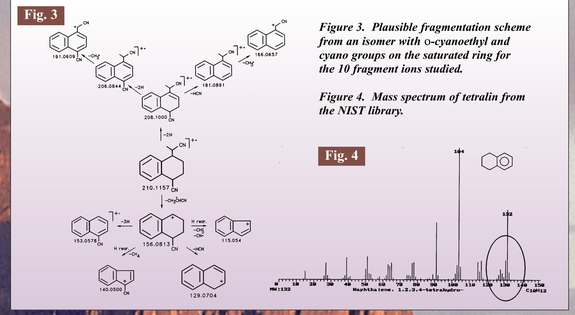


Figure 3. Plausible fragmentation scheme from an isomer with o-cyanoethyl and cyano groups on the saturated ring for the 10 fragment ions studied.

Figure 4. Mass spectrum of tetralin from the NIST library.

Fig. 4

6. Limiting the Isomers Possible with Exact Masses of Fragment Ions and Neutral Losses

Exact masses were determined at 10,000 resolution for the 10 fragment ions labeled in Figure 1b and listed in Table 2. The exact masses of the neutral losses listed in Table 3 were determined by subtracting the exact masses of each fragment ion from the exact mass of the presumed molecular ion. For each entry, the observed mass errors in both tables were acceptable for only 1 combination of atoms that contained the elements in the presumed molecular ion. One or two other possibilities were eliminated for 8 of the fragment ions and for the 8 corresponding neutral losses. The correlation of the neutral losses and the fragment ions was consistent with the assumption that $C_{14}H_{14}N_2^{+\bullet}$ was the molecular ion.

The exact masses of the fragment ions and neutral losses eliminated uncertainty about the number of N atoms in the fragments. For example, the composition of the ion corresponding to the base peak at m/z 129 resulted from loss of $C_4H_5N_2$ and not of C_5H_7N or of C_6H_9. $C_{10}H_9^+$, not $C_9H_7N^+$ or $C_8H_5N_2^+$, was consistent with the aromatic substructure shown in Figure 3 that originates from non-nitrogen containing compounds such as indanes, indenes, and tetralin [3]. Hence, the nitrogen atoms were probably external to the rings. The nominal mass loss of 54 Da could correspond to C_4H_6, C_3H_4N, or $C_2H_2N_2$ leaving 2, 1, or 0 N atoms in the fragment ion. The exact mass of 54.0344 Da indicated loss of C_3H_4N with 2.5 rings-and-double bonds, corresponding to a radical cleavage, either as a composite loss of ($\bullet C_2H_3$ + HCN) or of the intact units, $\bullet CH_2CH_2CN$ or CH_3 HCN. The library mass spectrum in Figure 4 for tetralin shows sequential loss of several H atoms, similar to the losses observed for the m/z 156 ion in Figure 1b. The neutral loss masses in Table 3 verified that the difference between m/z 153 and m/z 156 was 3 H atoms. After a tetralin core was assumed, 9 rings-and-double bonds required that the 2 N atoms be present as cyano groups. The loss of C_3H_4N then corresponded to an alkylcyano group and the loss of CH_3N corresponded to loss of (2H + HCN). Knowledge of the correct neutral losses led quickly to a limited number of possible isomers, those which contained cyano and alkylcyano groups attached to a tetralin core. Organic chemical considerations of long-term stability in water and solubility were also consistent with this functional group hypothesis. Additional observations that supported this hypothesis will be presented in reference 4.

7. Searching the Literature for Industrial Processes that Produce Such Isomers

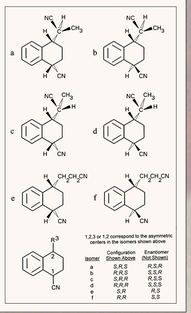
A search of the formula indices of Chemical Abstracts from 1920 to the present found hundreds of citations for compounds with the composition $C_{14}H_{14}N_2$. These were searched for industrial processes that could produce molecules with the expected structural features. Abstracts containing molecules shown in Figure 1c were located and the full papers were later reviewed. These compounds were produced by the copolymerization of styrene and acrylonitrile as 1:2 adducts, and had mass spectra very similar to those acquired for the isomers in the well water.

8. Establishing Responsibility for the Contaminants

Unknown to us at the time, the party who manufactured these chemicals admitted responsibility after we determined the elemental composition. The party later provided us an authentic standard obtained by distilling the adducts from styrene:acrylonitrile polymer that was prepared by another industrial synthesis. Three of the isomers observed in the standard had the same retention times as isomers in the well water extract and very similar mass spectra as illustrated by Figure 1. The possible structures of these isomers are shown in Figure 5.

Fig. 5

Figure 5. Six isomeric structures consistent with the experimental data and structural deductions of this study.



9. CONCLUSION

Several unidentified compounds present at trace levels in well water consumed by the public were found by the NJ Department of Environmental Protection. The Environmental Sciences Division of the EPA identified these isomeric compounds after determination of the elemental compositions of the molecular ion ($M^{+\bullet}$), 10 fragment ions, and the corresponding neutral losses.

Study of the molecular ion using MPPSIRD and the PGM established a unique elemental composition for the isomers in the well-water. The exact masses of the fragment ions and neutral losses, which were determined as the difference between the exact mass of the molecular ion and the fragment ions, greatly reduced the number of plausible structures for the isomers. This methodology of greatly reducing the number of possible isomers before searching the literature provided an efficient means for identifying the compounds. The data and interpretation presented above provided compelling evidence for establishing the source of the contaminants found in the well water.

10. REFERENCES

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